原　　著

Studies on Influence of Estrogen on Lipid Metabolism following Partial Resection of Liver

by

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Received for Publication Feb. 14, 1967

CONTENT

I. INTRODUCTION

Role of the liver in lipid metabolism is, as a matter of course, extremely important, and functional disorder of the liver is subtly reflected on lipid metabolism. Studies on disorder of lipid metabolism at hepatic insufficiency have been, however, concentrated mainly in clarifying pathology of fatty liver, and it has not been put so much emphasis from clinical aspects. It is assumed that this is principally due to the fact that lipid metabolism of the liver is so much complicated and diversified that pathophysiology of this organ is not yet well grasped.

Prosperous regeneration of the liver has been reported since early days. According to HIGGINS and ANDERSON[1], liver weight restores to preoperative value within 3 weeks in rats when 70 per cent of the liver is resected, and it is readily presumed that the phenomenon of liver regeneration is controlled and regulated by various hormones. It is a very interesting problem to study how lipids participate in such prosperous regeneration of the liver and how hormones of the sexual gland influence upon hepatic regeneration and lipid metabolism.

SZEGO[2] maintained that increase in liver weight in initial stage of regeneration after partial resection of the liver might be attributed to increase in lipids. AOYAMA[3] also asserted that lipids actively participate in prosperous regeneration of the liver and lipids are indispensable factor for the process of favorable regeneration. On the other hand, BENGMARK[4], SEKI[5] and other investigators observed that administration of testosterone propionate enhances regeneration of residual liver parenchyma and inhibits fatty infiltration in the liver after partial resection of the liver, and they considered this might be due to anabolic effect of this hormone. In this respect, it is difficult to assume that increase in hepatic fat in initial stage of liver regeneration can be interpreted simply as a manifestation of increase in regenerative function of the liver.

Present experiments were carried out to study influence of estrogen on hepatic regeneration and lipid metabolism following partial resection of the liver under the administration of estrogen which is in a particularly close relation with lipid metabolism among various hormones of the sexual gland.

II. MATERIALS AND METHODS

1. Materials

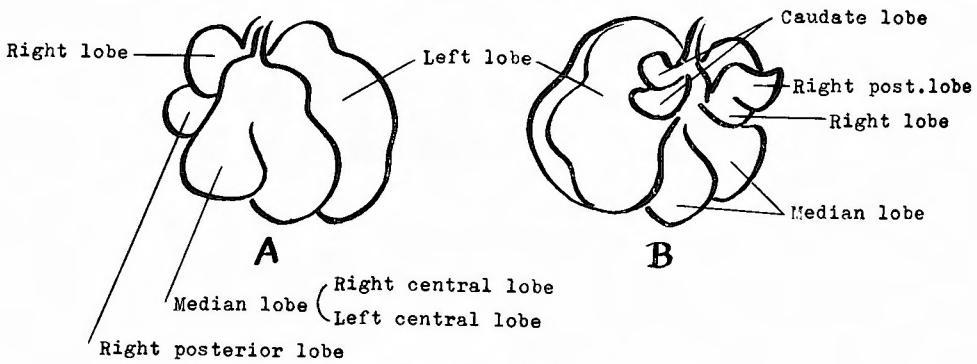
(1) Experimental Animal

Male rats of Saitama strain weighing from 100 to 160 g were used after housing at least for 10 days with adequate water and Oriental Solid Diet MF (product of Oriental Kobo Kogyo Co.).

(2) Anatomy of Rat Liver

The liver of rat is lobulated as shown in Fig. 1, being consisted of the median lobe (further lobulated into the right and left central lobes), right lobe, right posterior lobe behind, huge left lobe moreover and 2 caudate lobes of small size.

Fig. 1 Anatomy of the rat liver.



(A) seen from in front ; (B) seen from behind.

(3) Partial Resection of Liver

Procedure of partial resection of the liver was carried out after the method of HIGGINS and ANDERSON[1]. The abdomen was opened aseptically under ether anesthesia. The median and left lobes were resected with massive ligature. As shown in Tab. 1, average proportion of liver weight to body weight in 10 normal rats was 4.72 per cent, and, resected liver parenchyma corresponded to 62.0 per cent of the entire liver. Although

Table 1. Proportion of weight of liver to that of body and proportion of weight of removed liver to that of total liver at the time of partial hepatectomy in normal rats.

Rat No.	Body weight (g)	Total liver weight (g)	Proportion of weight of liver to that of body (%)	At the time of partial hepatectomy	
				Liver removed (g)	Proportion to total liver weight (%)
1	98	5.5	5.61	3.6	65.5
2	110	5.6	5.09	3 5	62.5
3	112	5.7	5.08	3.5	61.4
4	112	4.7	4.19	2.8	59.4
5	104	5.6	5.38	3.6	64.2
6	124	5.3	4.27	3.5	66.0
7	134	6.8	5.07	4.0	58.7
8	137	6.9	5.04	4.5	67.0
9	170	7.1	4.18	4.4	62.0
10	152	7.8	4.47	5.0	64.1
Average per 100 g. body weight			4.72%		62.0%

there exists some deviation of the value due to differences in body weight of experimental animals, it seems that this average value coincides with those values reported by HIGGINS and ANDERSON[1] to be 3.58 per cent of proportion of liver weight to body weight and 70.6 per cent of resected proportion to the entire liver, those by BRUES[6] to be 4.14 per cent and 68.4 per cent, those by HARKNESS[7] to be 3.47 per cent and 63.4 per cent and those by RABINOVICI[8] to be 3.80 per cent and 70.0 per cent, respectively. The experimental animals were given 20 per cent glucose solution alone on the day of operation, and housed with water and Oriental Solid Diet MF thereafter.

After partial resection of the liver, animals were slaughtered by heart puncture with the lapse of time of 10 hours, 1 day, 2 days, 3 days, 5 days, 7 days and 14 days respectively after surgery, and the liver was extirpated which was immediately subjected to the examinations.

(4) Estrogen

As estrogen, Ovahormone Benzoate Suspension, i.e. 1, 3, 5, (10)-estratriene-3, 17β-diol-3-monobenzoate (product of Teikoku Zoki Co.) was used. Based on the report of MIESCHER[9] that estrogen shows the maximum effect 8 days after the 1st injection in rats and the effect is maintained for 20 days, in the present experiment, 10μg of estrogen per 100 g of body weight was injected once intramuscularly and 7 days later partial resection of the liver was performed.

2. Methods

(1) Fluctuation of Body Weight

The rate of increase in body weight after partial resection of the liver was measured both in group of estrogen administration and that without the administration with the lapse of time of 10 hours, 1 day, 2 days, 3 days, 5 days, 7 days and 14 days after surgery, respectively. For the control study, a group of animals undergone simple laparotomy similarly under ether anesthesia was prepared, and the study was carried out in the following 4 groups of animals.

a) Group of simple laparotomy with estrogen administration

b) Group of simple laparotomy without estrogen administration

c) Group of partial resection of the liver with estrogen administration

d) Group of partial resection of the liver without estrogen administration

(2) Fluctuation of Liver Weight

Wet liver weight per 100 g of body weight was measured both in animals with and without estrogen administration with the lapse of time of 10 hours, 1 day, 2 days, 3 days, 5 days, 7 days and 14 days after partial resection of the liver.

(3) Histological Study

Slice sections of each regenerated liver from both animals with and without estrogen administration were fixed in a 20 per cent formalin solution and stained with hematoxylin and eosin for histological study.

(4) Determination of Hepatic Lipids

Both animals with and without estrogen administration were slaughtered by heart puncture 10 hours, 1 day, 2 days, 3 days and 5 days after partial resection of the liver, and the liver was extirpated and subjected immediately to determination of lipids in the liver. The blood obtained at heart puncture was used for determination of lipids in blood.

a) Total phospholipids were determined by method of FISKE and SUBBARAW[10] after extraction by FOLCH's method[11], and obtained results were multiplied by 25[12].

b) Total and free type cholesterols were determined by method of ZAK-KILLIANI[13] after extraction by FOLCH's method, and cholesterol of ester type was determined as the difference between total cholesterol value and free type cholesterol value.

c) Total fatty acids were determined by method of VAN de KAMER[14].

d) Neutral fat and total lipids were calculated from the formula of STAMLER[15][16].

(5) Determination of Lipids in Blood

a) Total phospholipids were determined by method of FISKE and SUBBARAW[11], and obtained results were multiplied by 25[12].

b) Total and free type cholerterols were determined by method of ZAK-KILLIANI[13], and cholesterol of ester type was determined as the difference between total cholesterol level and free type cholesterol level.

c) Total fatty acids were determined by method of STERN and SHAPIRO[17].

d) Neutral fat and total lipids were calculated from the formula of STAMLER[15][16].

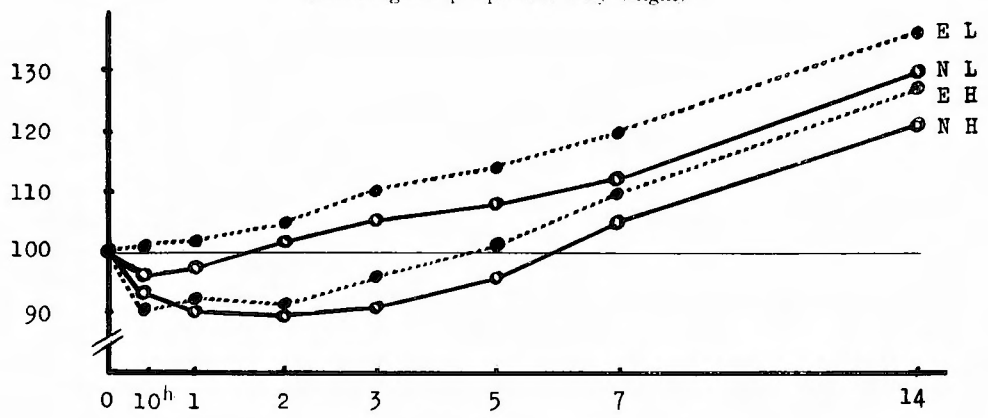
e) Non-esterified fatty acid (abbreviated to N. E. F. A., hereafter) was determined by modified method of DOLE[18][19].

III. RESULTS

1. Increase in Body Weight following Partial Resection of Liver

The rate of increase in body weight following partial resection of the liver in rats was studied in comparison with the fluctuation of body weight in animals of simple laparotomy for control study. Postoperative fluctuation of body weight is shown in Fig. 2 in the term of percentage of preoperative body weight. In the group of simple laparotomy, decrease in body weight was extremely slight and it restored within a short period, whereas in the group of partial resection of the liver, it decreased abruptly in an early postoperative stage, reaching 90 per cent of the preoperative value 2 days after surgery,

Fig. 2 Increase in body weight for 14 days in simply laparotomized rats and partially hepatectomized rats. (Percentage of preoperative body weight)



E L : Estrogen-treated, simply laparotomized series
N L : Non-treated, simply laparotomized series
E H : Estrogen-treated, partially hepatectomized series
N H : Non-treated, partially hepatectomized series

which gradually increased thereafter to restore to preoperative value from 5 to 7 days after surgery. In the group of simple laparotomy with estrogen administration, postoperative body weight loss could not be observed at all, and the increase in body weight was more rapid in the group of partial resection of the liver with administration of estrogen than in the group of partial resection of the liver without administration of estrogen.

2. Increase in Liver Weight following Partial Resection of Liver

Fluctuation of wet liver weight per 100 g of body weight in rats after partial resection of the liver is shown in Fig. 3. After partial resection of the liver, the residual liver parenchyma showed extremely prosperous hypertrophic regeneration, and liver weight increased rapidly until 3rd day after surgery. However, increase in liver weight became more mild later than 3rd day after surgery. These findings are accepted to coincide so well with the reports of HIGGINS and ANDERSON[1], SZEGO[2], AOYAMA[3], BENGMARK[4], BRUES[6] and other investigators that the maximum regeneration rate could be observed 2 or 3 days after surgery. As obviously understood from Fig. 3, rate of increase in liver weight in the group of estrogen administration was so large as compared with that in the group without estrogen administration, and difference in rate of increase in liver weight in these two groups was particularly large 2 days after partial resection of the liver when regeneration of the residual liver parenchyma was most prosperous.

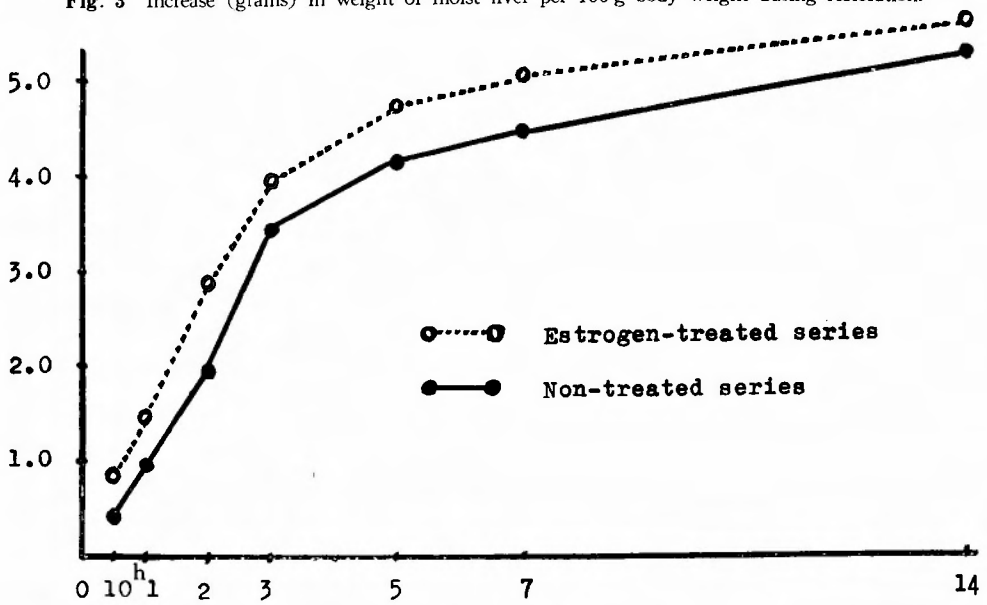
3. Histological Findings of Regenerated Liver

There was little significant difference in histological findings of regenerated liver tissue of both groups with and without estrogen administration.

a) Ten hours after partial resection of the liver (Fig. 25), hyperemia and hemorrhage in the sinusoids, diffuse swelling of the hepatic cells and vacuole formation are observed.

b) One day after partial resection of the liver (Fig. 26), hyperemia and hemorrhage, irregularity of the hepatic cell cord, cloudy swelling and vacuole formation are remarkably

Fig. 3 Increase (grams) in weight of moist liver per 100 g body weight during restoration.



observed.

c) Two days after partial resection of the liver (Fig. 27), hyperemia and irregularity of the hepatic cell cord are observed to be slight. Hepatic cells are relatively large with conspicuously frequent mitotic figures.

4. Changes of Hepatic Lipids following Partial Resection of the Liver

Since there exists considerable wide range of physiological fluctuation of intrahepatic lipid content, it is difficult to establish strict border line between normal and pathologic ranges. BEST[20] reported normal range of hepatic lipid content in men to be 5 to 6 per cent, and according to DUEL[21] it ranges from 3 to 5 per cent. Concerning total lipid content in the liver of rats, BENGMARK[4] reported it to be 6.2 per cent, HARKNESS[7] to be 3.3 per cent and MATSUOKA[22] to be 3.77 per cent. In the present experiment, total lipid content was 6.08 ± 0.70 per cent of wet liver tissue. Average values of each fraction of hepatic lipids before operation in the groups with and without estrogen administration are shown in Tab. 2. In total hepatic lipids in the group without estrogen administration, total phospholipids were 50.4 per cent, total cholesterol was 20.5 per cent (free type being 15.3 per cent and ester type being 5.2 per cent) and the remainder was consisted of neutral fat. Postoperative changes of each fraction of hepatic lipids are summarized in Tab. 3 and 4. Experimental results of each fraction are described in details in the following.

a) Total Phospholipids

As shown in Fig. 4, total phospholipids reached its maximum level 10 hours after partial resection of the liver in the groups both with and without estrogen administration, which restored to preoperative level 2 to 3 days after surgery. There was no significant difference in these two groups.

b) Cholesterol

Total cholesterol showed its peak from 10 hours to 1 day after surgery, and decreased approximately to preoperative level 5 days after surgery (Fig. 5). As shown in Fig. 6, cholesterol of free type showed little change throughout pre- and postoperative periods. Cholesterol of ester type increased remarkably, as in Fig. 7, 10 hours after surgery, and it restored to preoperative level 3 days after surgery. As described here, it is worth while to note that intrahepatic cholesterol in the group with estrogen administration showed

Table 2. Average liver lipid values of estrogen-treated rats and non-treated rats, shown as g/100g wet liver tissue.

	Estrogen-treated rats ( )... No. of rats	Non-treated rats ( )... No. of rats
Total phospholipids	3.07 ± 0.39 (9)	3.07 ± 0.42 (9)
Total cholesterol	1.17 ± 0.28 (8)	1.25 ± 0.24 (9)
Free cholesterol	0.87 ± 0.22 (8)	0.93 ± 0.17 (9)
Esterified cholesterol	0.30 ± 0.05 (8)	0.32 ± 0.02 (9)
Total fatty acids	4.00 ± 0.44 (6)	4.18 ± 0.68 (8)
Neutral fat	1.80 ± 0.41 (7)	2.08 ± 0.55 (9)
Total lipids	6.00 ± 0.31 (7)	6.08 ± 0.70 (9)
Cholesterol-ester ratio	25.6 ± 2.80 %	25.6 ± 2.73 %
Total cholesterol/total phospholipids ratio	41.6 ± 4.25 %	41.7 ± 4.30 %

Table 3. Average liver lipid values of non-treated, partially hepatectomized rats, shown as g/100g wet liver tissue.

	Controls	Time in days after operation				
		10 hr.	1	2	3	5
TP	3.07±0.42 (100)	3.85±0.50 (125)	3.28±0.41 (107)	3.00±0.47 (97)	3.03±0.30 (98)	3.05±0.30 (99)
TC	1.25±0.24 (100)	1.73±0.30 (138)	1.72±0.37 (129)	1.45±0.31 (113)	1.30±0.10 (104)	1.14±0.33 (91)
FC	0.93±0.17 (100)	1.04±0.17 (111)	1.08±0.26 (116)	1.00±0.20 (107)	0.99±0.10 (106)	0.85±0.24 (91)
EC	0.32±0.02 (100)	0.69±0.14 (216)	0.64±0.14 (200)	0.45±0.10 (140)	0.31±0.05 (97)	0.29±0.06 (91)
TFA	4.18±0.68 (100)	15.03±2.79 (358)	10.28±2.51 (245)	7.45±1.61 (177)	5.12±0.53 (121)	4.66±0.62 (110)
NF	2.08±0.55 (100)	12.56±2.58 (603)	8.18±2.73 (389)	5.30±1.70 (252)	3.02±0.63 (143)	2.76±0.66 (131)
TL	6.08±0.70 (100)	17.73±3.28 (290)	12.58±2.58 (206)	9.75±1.61 (160)	7.07±0.70 (116)	6.76±0.63 (110)
EC/TC (%)	25.6±2.73 (100)	39.8±4.52 (155)	37.2±4.00 (145)	31.0±4.15 (121)	23.8±2.70 (93)	25.4±2.86 (99)
TC/TP (%)	41.7±4.30 (100)	44.8±4.68 (107)	52.4±5.80 (126)	48.2±5.00 (116)	43.2±4.41 (104)	37.8±4.12 (91)

Values in brackets represent percentage changes.
TP : Total phospholipids. TC : Total cholesterol. FC : Free cholesterol.
EC : Esterified cholesterol. TFA : Total fatty acids. NF : Neutral fat.
TL : Total lipids.

Table 4. Average liver lipid values of estrogen-treated, partially hepatectomized rats, shown as g/100g wet liver tissue.

	Controls	Time in days after operation				
		10 hr.	1	2	3	5
TP	3.07±0.31 (100)	3.45±0.37 (112)	3.05±0.34 (99)	3.06±0.36 (99)	3.08±0.33 (101)	2.97±0.38 (96)
TC	1.17±0.28 (100)	1.47±0.20 (125)	1.50±0.37 (128)	1.36±0.26 (116)	1.30±0.24 (111)	1.15±0.20 (98)
FC	0.87±0.22 (100)	0.87±0.17 (100)	0.95±0.24 (109)	0.94±0.17 (108)	0.94±0.17 (108)	0.85±0.14 (97)
EC	0.30±0.05 (100)	0.60±0.11 (200)	0.55±0.05 (183)	0.42±0.08 (140)	0.36±0.06 (120)	0.30±0.06 (100)
TFA	4.00±0.44 (100)	8.32±0.61 (208)	7.81±0.81 (195)	5.00±1.22 (124)	4.00±0.63 (100)	4.16±0.44 (103)
NF	1.80±0.41 (100)	5.93±0.67 (328)	5.81±0.77 (322)	3.01±1.18 (167)	2.01±0.56 (111)	2.13±0.52 (118)
TL	6.00±0.31 (100)	10.82±0.31 (180)	10.25±0.63 (170)	7.40±1.34 (123)	6.30±0.70 (105)	6.13±0.63 (102)
EC/TC (%)	25.6±4.25 (100)	40.0±6.40 (156)	36.7±4.43 (143)	30.2±3.51 (118)	28.0±3.33 (109)	26.5±4.31 (103)
TC/TP (%)	41.6±4.25 (100)	42.6±5.10 (102)	49.5±5.90 (119)	45.1±5.34 (108)	42.0±4.07 (101)	38.1±4.30 (91)

Values in brackets represent percentage changes.
TP : Total phospholipids. TC : Total cholesterol. FC : Free cholesterol.
EC : Esterified cholesterol. TFA : Total fatty acids. NF : Neutral fat.
TL : Total lipids.

Fig. 4 Change in total phospholipids (g/100 g wet liver tissue)

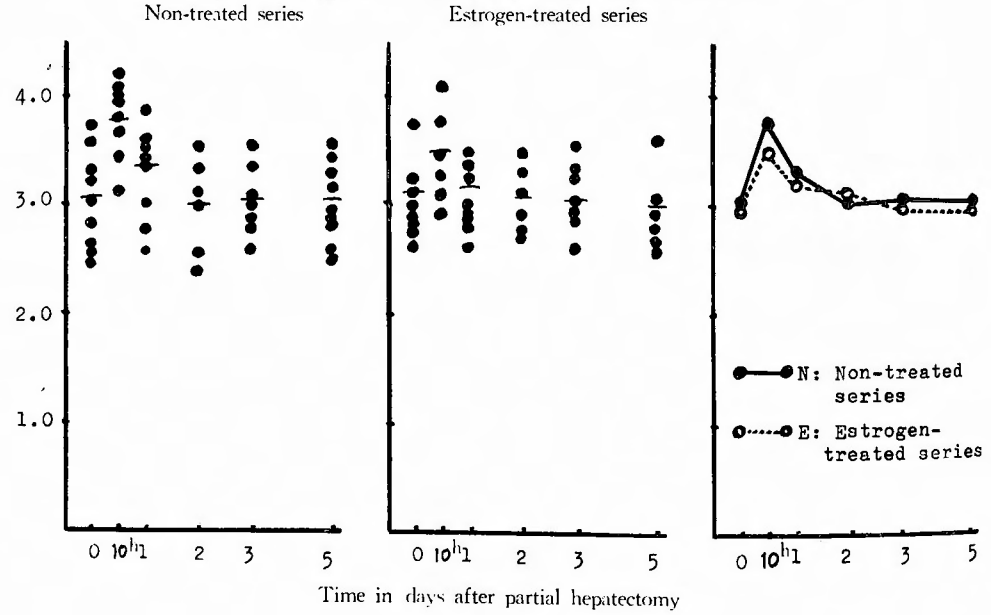
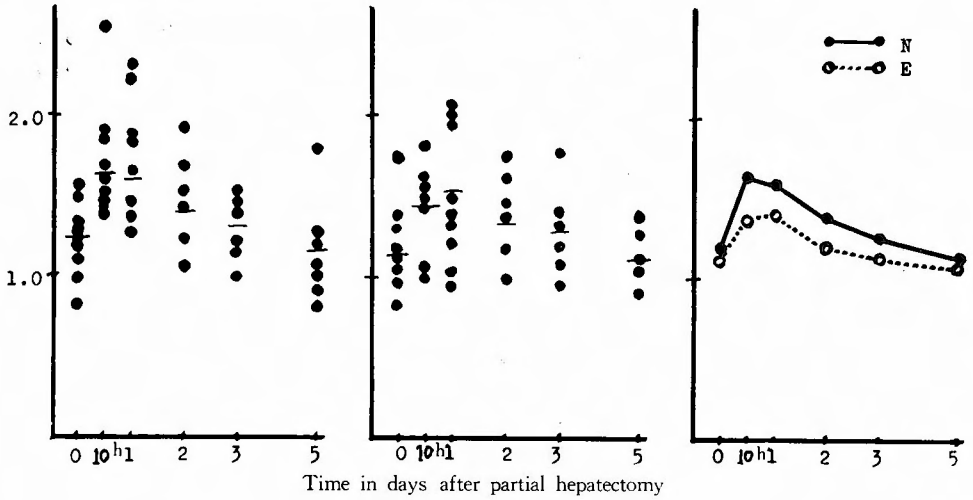
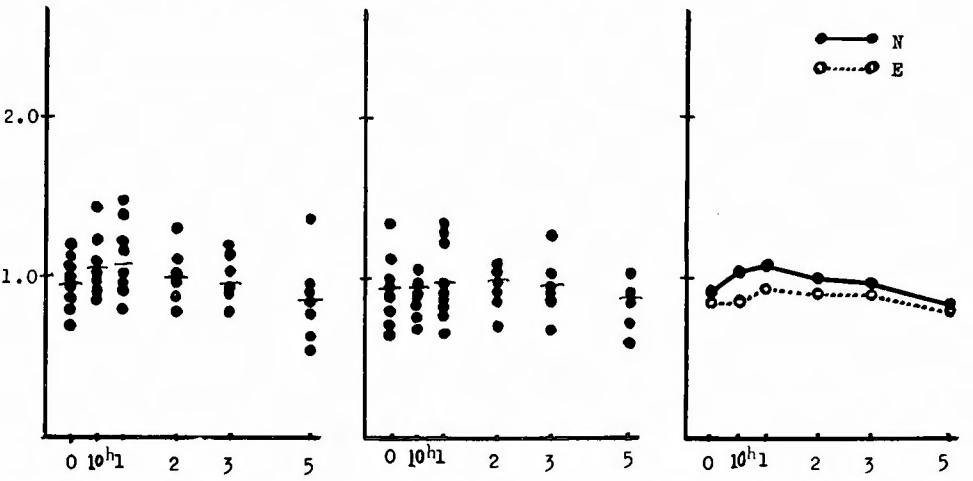


Fig. 5 Change in total cholesterol (g/100 g wet liver tissue)
Non-treated series — — Estrogen-treated series — — —



Time in days after partial hepatectomy

Fig. 6 Change in free cholesterol (g/100 g wet liver tissue)
Non-treated series Estrogen-treated series



Time in days after partial hepatectomy

lower value than in the group without estrogen administration almost throughout the entire course of the experiment. Cholesterol ester ratio showed the maximum value 10 hours after surgery, which decreased gradually thereafter, showing little significant difference between these two groups (Fig. 8).

c) Total Cholesterol/Total Phospholipids Ratio

As shown in Fig. 9, total cholesterol/total phospholipids ratio showed the maximum level 1 day after surgery in both groups with and without estrogen administration, which was followed by gradual decrease thereafter, revealing little significant difference between these two groups.

d) Total Fatty Acids

Fig. 7 Change in esterified cholesterol (g/100 g wet liver tissue)

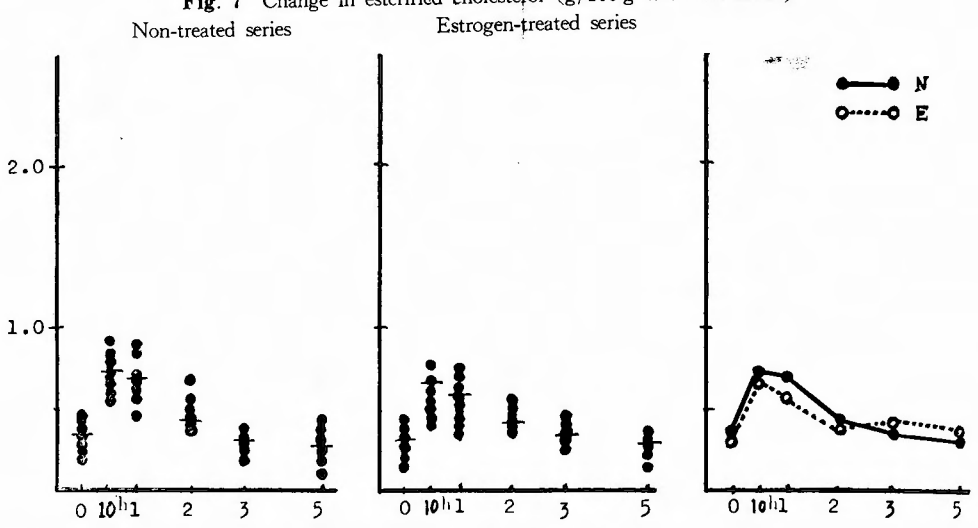


Fig. 8 Change in cholesterol-ester ratio (%)

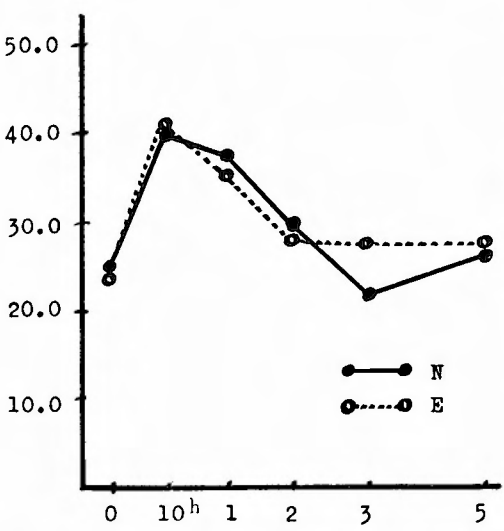
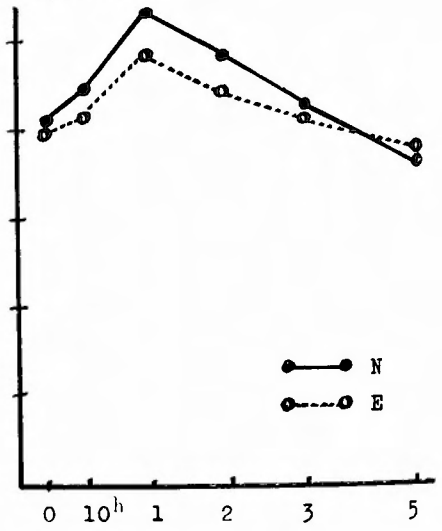


Fig. 9 Change in total cholesterol/total phospholipids ratio (%)



Time in days after partial hepatectomy

As shown in Fig. 10, total fatty acids showed such a marked increase as 3.58 times of preoperative value 10 hours after partial resection of the liver in the group without estrogen administration. In the group with estrogen administration, total fatty acids showed considerably lower level compared with the group without estrogen administration, and restoration of total fatty acid level was more rapid in the latter group.

e) Neutral Fat

In contrast to the fact that among intrahepatic lipids, phospholipids and cholesterol showed relatively slight changes, neutral fat content fluctuated readily to cause so-called

fatty liver. Intrahepatic neutral fat showed the maximum level 10 hours after partial resection of the liver, as shown in Fig. 11, and particularly in the group without estrogen administration, and the increase was so large as 6 times of preoperative level, ranging 12.56 ± 2.58 g per 100 g of wet liver weight. On the other hand, neutral fat in the group with estrogen administration was 5.93 ± 0.67 g per 100 g of wet liver weight even at the time of the maximum increase, revealing small value as 50 per cent of the value in the group without estrogen administration.

f) Total Lipids

As reported by Naito[16] that total fatty acids, neutral fat and total lipids fluctuate frequently in parallel with each other, these three components showed similar fluctuation in the present experiment. Intrahepatic total lipids showed the maximum level 10 hours

Fig. 10 Change in total fatty acids (g/100g wet liver tissue)

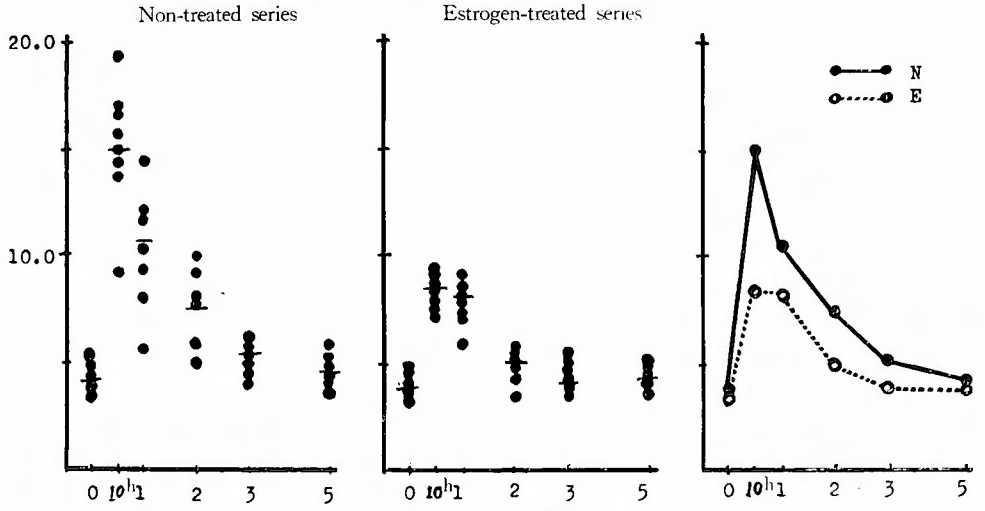


Fig. 11 Change in neutral fat (g/100 g wet liver tissue)

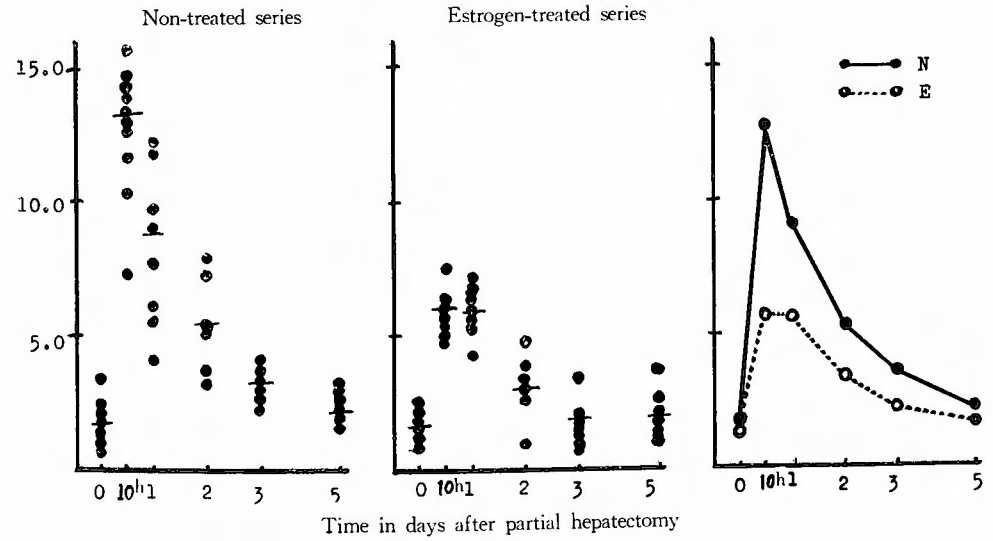
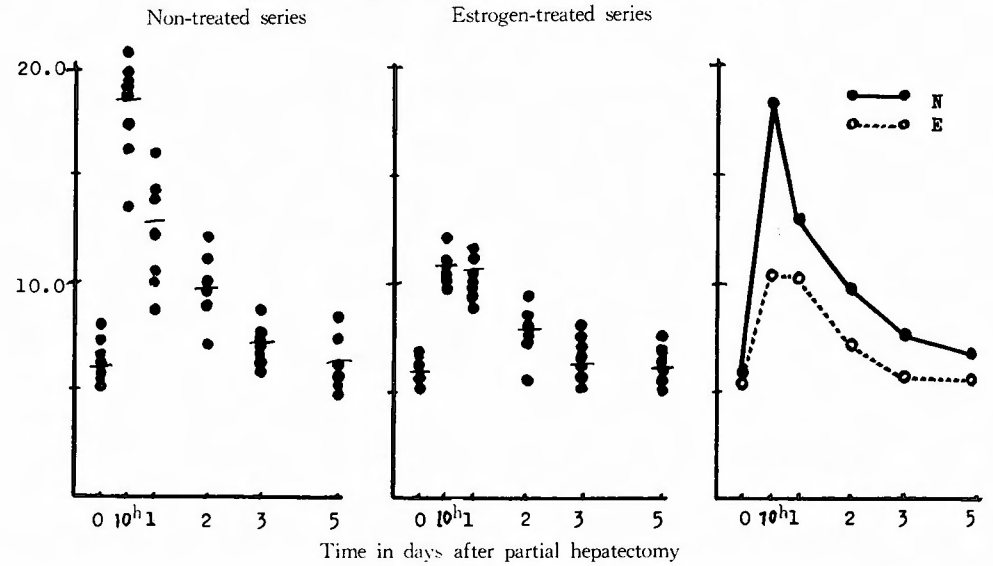


Fig. 12 Change in total lipids (g/100 g wet liver tissue)



after surgery in both groups with and without estrogen administration, as shown in Fig. 12, which was followed by decrease. It is an interesting finding that total lipid level was constantly lower throughout the entire course of the experiment in the group with estrogen administration than in the group without estrogen administration, as intrahepatic total fatty acid and neutral fat levels were.

5. Changes of Lipids in Blood following Partial Resection of Liver

Pre- and postoperative changes of each fraction of lipids in blood in both groups with and without estrogen administration are summarized in Tab. 5 and 6. Experimental results of each fraction are described in details in the following.

Fig. 13 Change in total phospholipids (mg/dl serum)

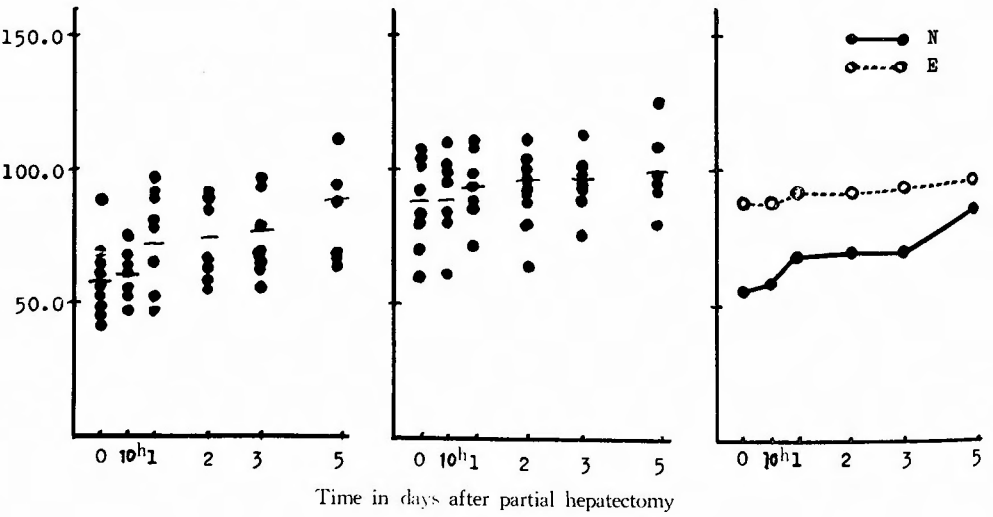


Table 5. Average serum lipid values of non-treated, partially hepatectomized rats, shown as mg/dl serum.

	Controls	Time in days after operation				
		10 hr.	1	2	3	5
TP	55.6±11.5 (100)	59.9±14.4 (107)	69.6±18.5 (124)	70.8±9.80 (128)	72.3±12.6 (130)	81.8±20.4 (147)
TC	68.4±10.1 (100)	58.6±10.0 (86)	52.6±12.8 (77)	52.0±17.7 (76)	50.7±17.0 (71)	50.3±10.4 (73)
FC	17.3± 3.0 (100)	20.1± 4.7 (116)	18.3± 5.3 (106)	20.2± 7.0 (117)	17.2± 6.2 (99)	16.7± 1.2 (96)
EC	51.1± 6.7 (100)	38.5± 8.4 (75)	34.3± 9.2 (67)	31.8± 1.1 (62)	33.6± 7.0 (66)	33.6± 6.2 (66)
TFA	94.7±27.0 (100)	99.0±28.6 (104)	98.0±23.1 (103)	96.0±30.5 (101)	91.2±38.2 (96)	89.8±19.8 (94)
NF	20.0± 5.1 (100)	31.3± 7.6 (160)	30.8± 5.8 (150)	27.1± 7.4 (135)	18.8± 8.1 (94)	10.4± 3.1 (52)
TL	178.5±43.5 (100)	178.6±42.1 (100)	175.0±37.4 (98)	175.±51.7 (98)	165.0±66.7 (92)	164.2±42.6 (91)
NEFA (μEq/L)	550 ± 104 (100)	607 ± 114 (110)	607 ± 104 (110)	703 ± 118 (129)	767 ± 154 (138)	804 ± 170 (146)
EC/TC(%)	71.7±21.0 (100)	65.7±17.0 (87)	65.0±16.3 (87)	60.0±13.9 (80)	67.0±15.1 (89)	67.2±15.8 (90)
TC/TP(%)	123.4±33.1 (100)	97.2±21.4 (79)	76.0±16.0 (61)	73.1±15.9 (59)	69.8±14.2 (56)	60.9±13.7 (49)

Values in brackets represent percentage changes.
TP : Total phospholipids. TC : Total cholesterol. FC : Free cholesterol.
EC : Esterified cholesterol. TFA : Total fatty acids. NF : Neutral fat.
TL : Total lipids. NEFA : Non-esterified fatty acid

Fig. 14 Change in total cholesterol (mg/dl serum)

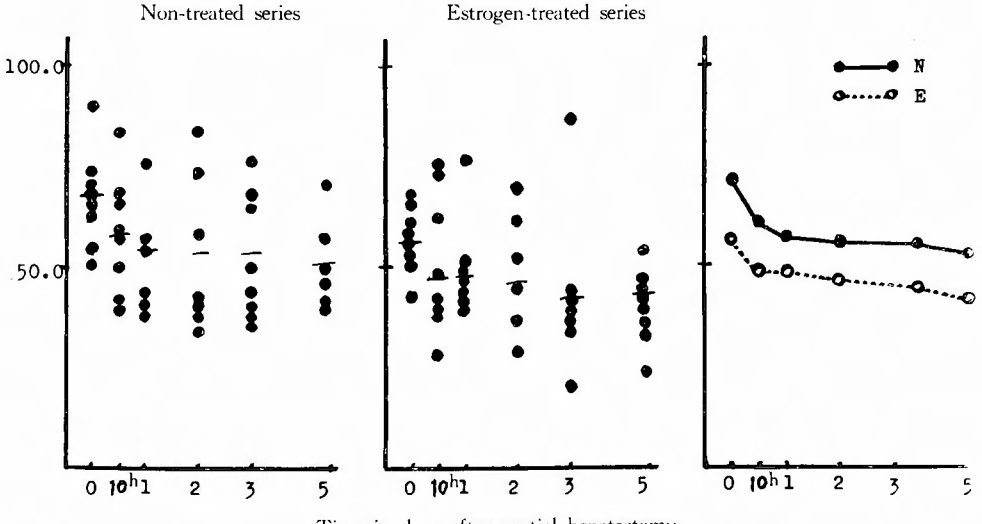


Table 6. Average serum lipid values of estrogen-treated, partially hepatectomized rats, shown as mg/dl serum.

	Controls	Time in days after operation				
		10 hr.	1	2	3	5
TP	85.8±18.7 (100)	86.3± 5.2 (102)	89.8±17.6 (104)	90.7±11.4 (105)	93.2±11.1 (108)	97.3±17.3 (112)
TC	57.3± 7.2 (100)	48.0±18.1 (84)	47.7±12.7 (83)	47.0±15.3 (83)	42.5±20.6 (75)	41.6± 9.0 (73)
FC	14.4± 2.0 (100)	16.8± 6.1 (116)	16.5± 4.7 (115)	18.6± 6.4 (129)	14.1± 6.9 (98)	13.6± 2.8 (94)
EC	42.9± 4.1 (100)	31.2± 9.2 (72)	31.2± 8.0 (72)	28.4± 5.1 (66)	28.4± 8.9 (66)	28.0± 7.0 (65)
TFA	129.1±26.2 (100)	135.0±41.2 (101)	127.8±38.4 (99)	135.0±27.3 (101)	131.2±21.9 (101)	129.7±40.2 (100)
NF	41.0± 9.9 (100)	54.8±18.1 (133)	45.3±13.4 (110)	53.6±11.2 (130)	49.0± 7.4 (119)	44.7± 7.3 (109)
TL	216.9±50.9 (100)	215.1±73.4 (99)	210.7±70.0 (101)	207.0±50.5 (97)	205.2±58.7 (96)	204.1±52.7 (95)
NEFA (μEq/L)	462 ± 109 (100)	490 ± 89 (106)	506 ± 134 (110)	574 ± 141 (125)	627 ± 141 (136)	651 ± 94 (141)
EC/TC(%)	75.4± 8.2 (100)	64.3± 7.1 (85)	64.5± 6.9 (85)	59.5± 6.5 (79)	65.1± 7.0 (86)	69.3± 7.7 (90)
TC/TP(%)	66.6± 7.2 (100)	55.8± 6.2 (84)	53.0± 5.9 (79)	52.8± 5.4 (79)	46.3± 5.0 (69)	43.3± 4.7 (65)

Values in brackets represent percentage changes.
TP : Total phospholipids. TC : Total cholesterol. FC : Free cholesterol.
EC : Esterified cholesterol. TFA : Total fatty acids. NF : Neutral fat.
TL : Total lipids. NEFA : Non-esterified fatty acid.

Fig. 15 Change in free cholesterol (mg/dl serum)

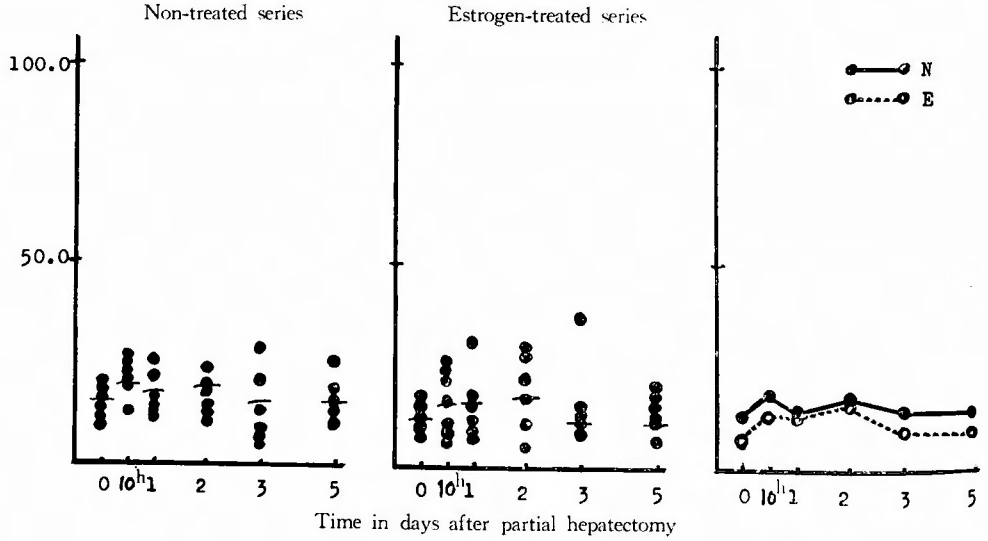


Fig. 16 Change in esterified cholesterol (mg/dl serum)

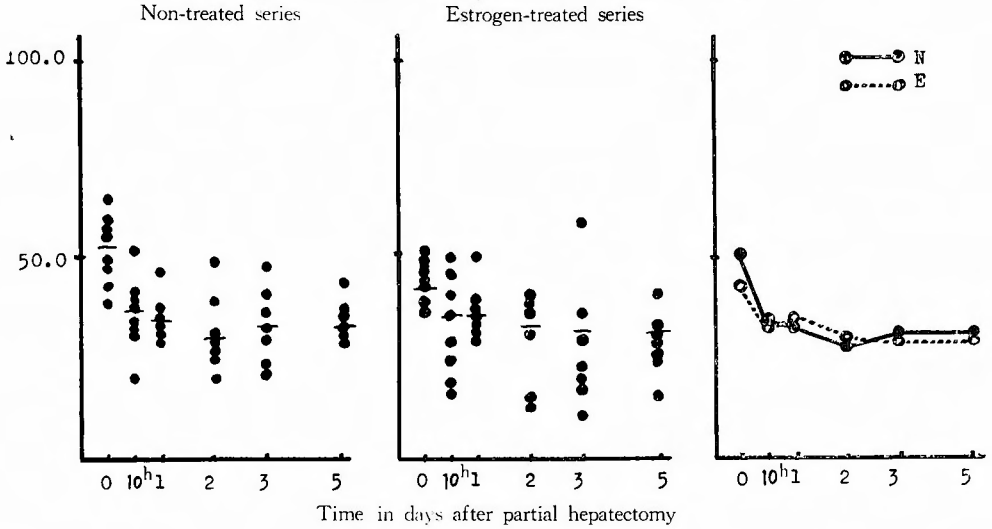
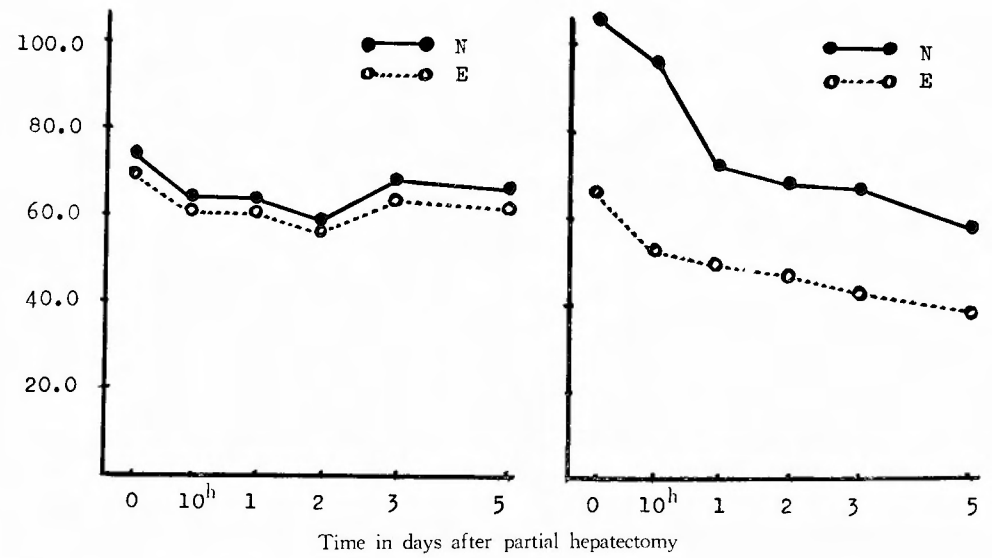


Fig. 17 Change in choesterol-ester ratio (%)

Fig. 18 Change in total cholesterol/total phospholipids ratio (%)



a) Total Phospholipids

Although there could be observed slight increase after partial resection of the liver in both groups with and without administration of estrogen, the former constantly showed higher blood phospholipid than the latter throughout the entire course of the experiment (Fig. 13).

b) Cholesterol

Total cholesterol showed a tendency of decrease after partial resection of the liver in both groups with and without estrogen administration, as shown in Fig. 14, and contrari-

Fig. 19 Change in total fatty acids (mg/dl serum)

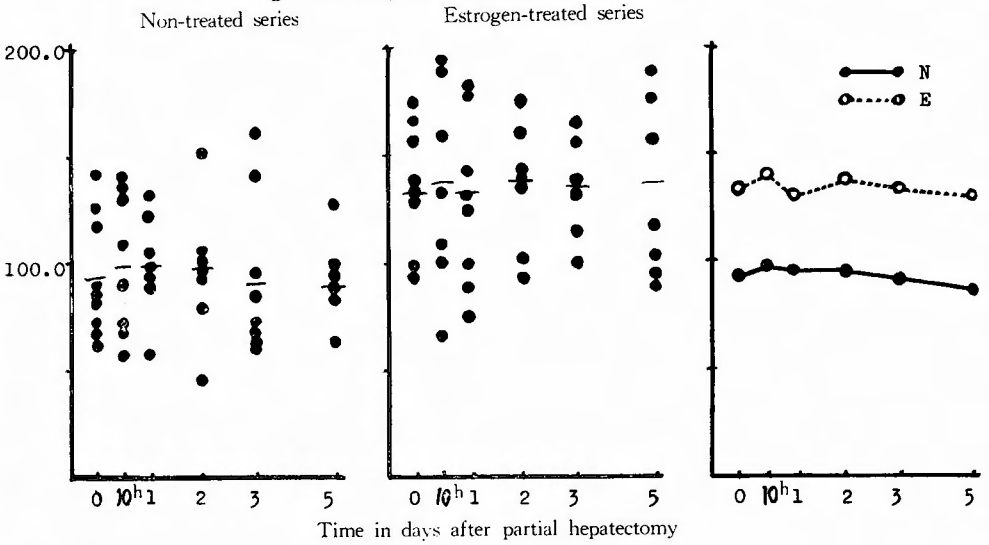
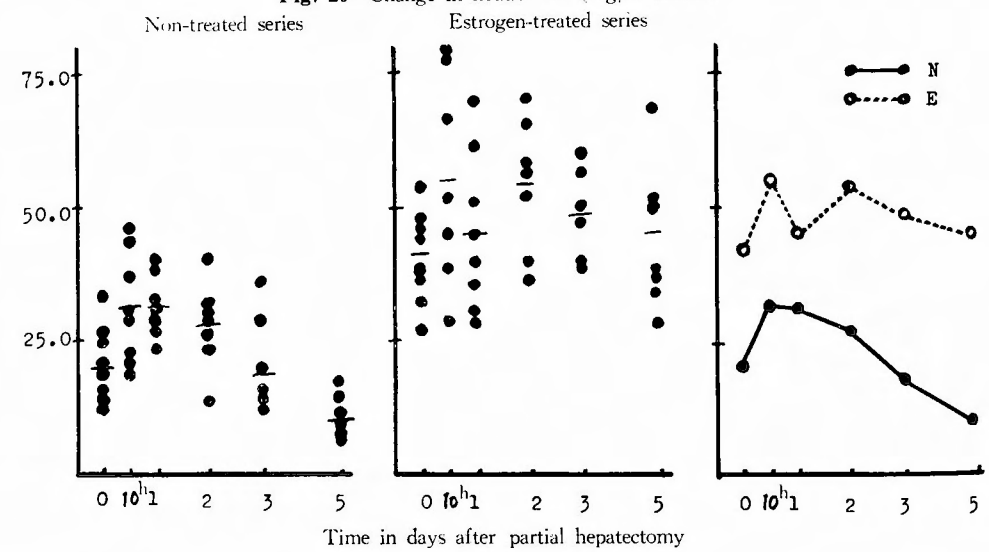


Fig. 20 Change in neutral fat (mg/dl serum)



wise to total phospholipids, cholesterol level in blood was constantly higher in the latter. Cholesterol of free type showed, as shown in Fig. 15, slight increase after surgery, with necessarily resulting decrease in esterified cholesterol (Fig. 16). Cholesterol-ester ratio was the smallest 2 days after surgery, and there was little significant difference between these two groups (Fig. 17).

c) Total Cholesterol/Total Phospholipids Ratio

As shown in Fig. 18, total cholesterol/total phospholipids ratio showed a tendency of decrease after partial resection of the liver in both groups with and without estrogen administration, particularly in the latter. Constantly smaller ratio of the group with estrogen

Fig 21 Changg in total lipids (mg/dl serum)

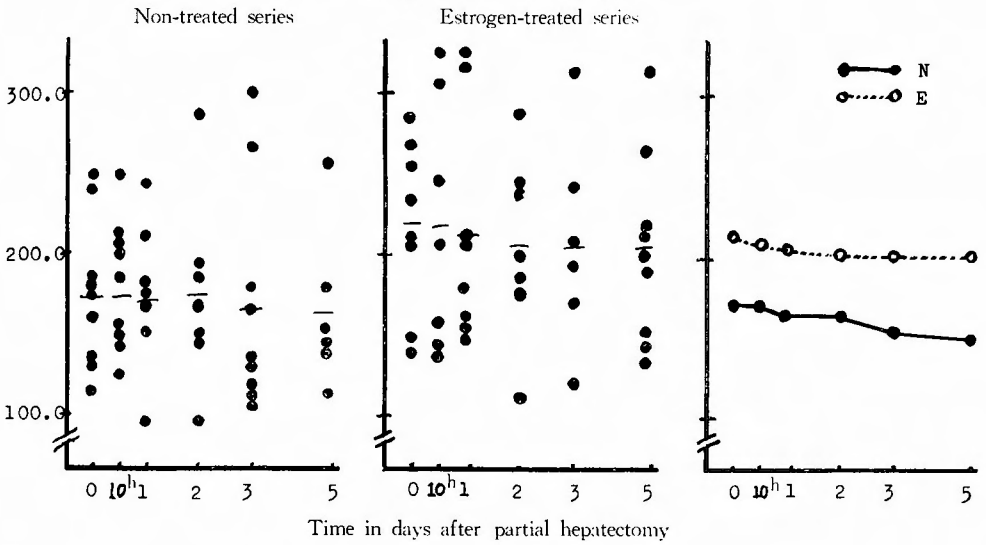
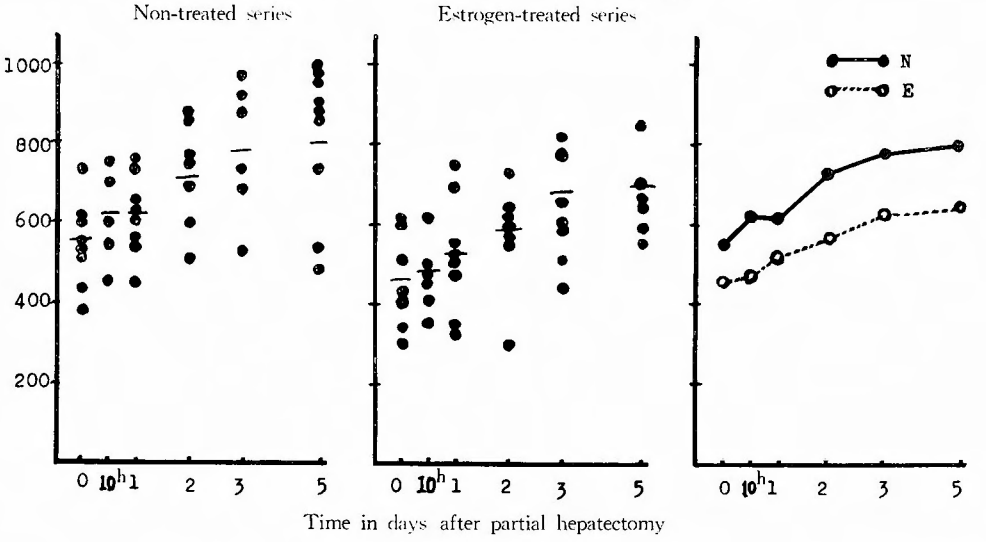


Fig. 22 Change in non-esterified fatty acid (μEq/L serum)



administration throughout the entire course of the experiment might be interpreted to coincide with the report of ANDO[23] that estrogen injection in male rats resulted in decrease in total cholesterol/total phospholipids ratio.

d) Total Fatty Acids

Average value of total fatty acids content in blood, which shows individual deviations in rats in a considerable degree, was invariably larger in the group with estrogen administration than in the group without estrogen administration, as shown in Fig. 19.

e) Neutral Fat

As shown in Fig. 20, neutral fat showed the maximum level 10 hours after partial

resection of the liver in both groups with and without estrogen administration, which restored to or below preoperative level from 3 to 5 days after surgery. It is worth while to note that neutral fat content was invariably higher in the group with estrogen administration than in the group without estrogen administration throughout the entire course of the experiment.

f) Total Lipids

Although there was extremely large deviations of the individuals, total lipids content in blood was larger, though slightly, in the group with estrogen administration than in the group without estrogen administration (Fig. 21).

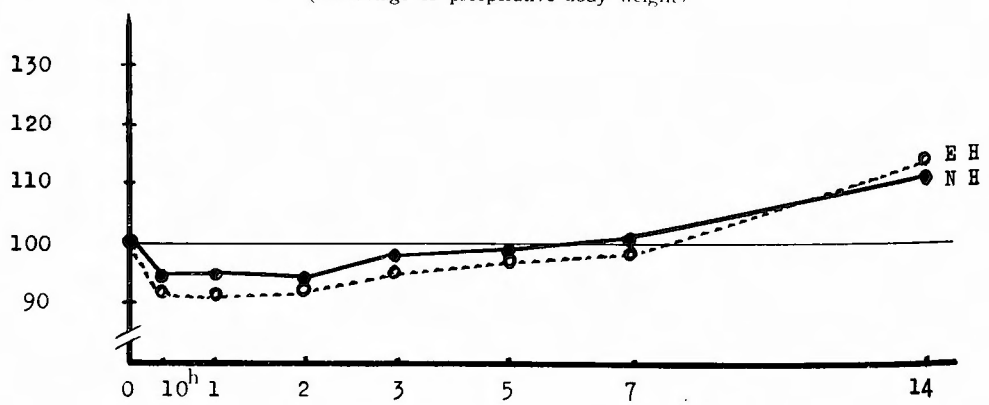
g) N. E. F. A.

Preoperative value of N. E. F. A. in the group with estrogen administration was $462 \pm 109\ \mu Eq/L$, as shown in Tab. 5 and 6, and $550 \pm 104\ \mu Eq/L$ in the group without estrogen administration. Thus, N.E.F.A. content in blood is extremely small. N.E.F.A. is, however, of utmost importance as a to-be-mobilized type of lipids, and numerous studies are being carried out on this subject. Although N. E. F. A. showed marked increase in both groups with and without estrogen administration after partial resection of the liver, as shown in Fig. 22, it is an extremely interesting finding that N. E. F. A. content in blood was constantly smaller in the group with estrogen administration than in the group without estrogen administration throughout the entire course of the experiment.

6. Increase in Body and Liver Weight in Female Rats following Partial Resection of Liver

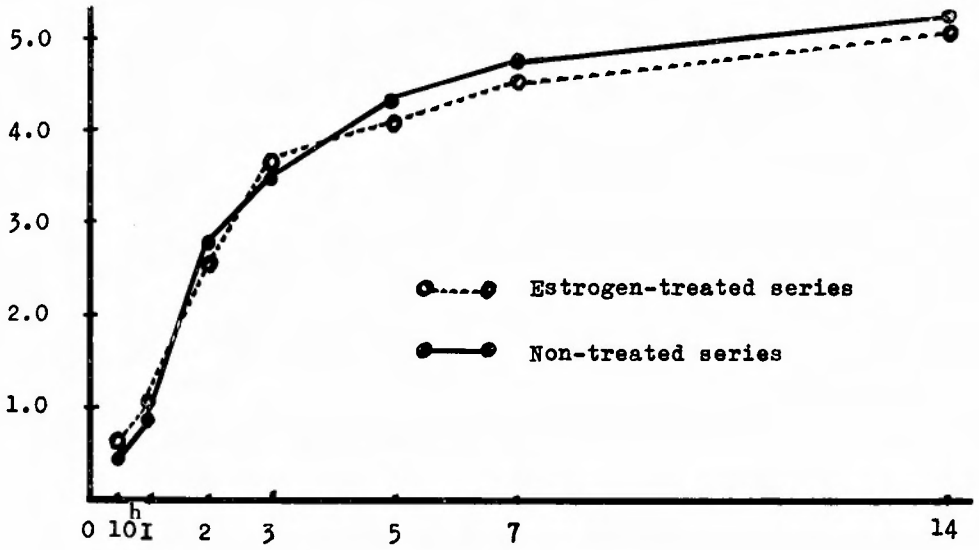
In the examination of fluctuation of body weight in female rats after partial resection of the liver, restoration of body weight to preoperative level was attained later than 7 days after surgery in both groups with and without estrogen administration, showing considerable retardation of the restoration as campared with that in male rats, as shown in Fig. 23.

Fig. 23 Increase in body weight for 14 days in partially hepatectomized female rats.
(Percentage of preoperative body weight)



Time in days after partial hepatectomy
EH : Estrogen-treated, partially hepatectomized rats
NH : Non-treated, partially hepatectomized rats

Fig. 24 Increase (grams) in weight of moist liver per 100 g body weight during restoration in female rats.



Increase and change in liver weight showed little significant difference between the group with and without estrogen administration, as in Fig. 24, showing similar tendency to the group of male rats without estrogen administration.

IV. DISCUSSION

The problem whether fatty liver which appears relatively acutely after partial resection of the liver can be deemed as an indicative factor of functional disorder of this organ or not, requires further investigations from various aspects. Here, the author of the present paper intends to make some considerations on this problem from the aspect of lipid metabolism.

After partial resection of the liver in rats, intrahepatic lipid content within the residual liver parenchyma increases from extremely early stage[3)4)6)7)24)25)]. This increase in intrahepatic lipids was explained by STETTEN[26)] to be a mobilization of lipids from depot fat to the liver. However, it is reported that the increase in intrahepatic lipid content can be well prevented by extirpation of the hypophysis[27)] or adrenal gland[24)25)]. Since ANSELMINO[28)] reported in 1931 that the extracts of the hypophysis of various animals caused an increase in lipid content of the liver in hamsters, it has been recognized that the extracts from the anterior[29)] or posterior pituitary[30)31)] or liver tissue itself[32)] contains certain factor which causes mobilization of neutral fat from depot fat, and SEIFTER[30)31)] called it lipid mobilizer. In mobilization of lipid from depot fat, it is presumed, any way, that hypophysis-adrenal system participates in this mechanism playing a very important role.

In recent years, role of the liver on lipids in blood has been clarified by tracer experiments using radioactive isotope and experiments employing animals whose livers are extirpated. Concerning phospholipid, namely, FISHLER[33)] demonstrated that phospholipid

to be synthesized from labelled non-organic phosphorus does not increase in dogs whose livers are extirpated. ENTENMAN[34] observed also that labelled phospholipid infused into dogs without liver is not eliminated outside the body. GOLDMAN[35], moreover, could not find out synthesized labelled phospholipid in serum, even if labelled palmitic acid was infused into dogs without liver. From these observations, it is obviously infered that phospholipid in blood originates in the liver and removed from blood in the liver. BALFOUR[36] interpreted fluctuation of phospholipid in blood as a reflection of activity of phospholipid synthesis. When synthesis of phospholipid is studied from the aspect of radioactive ^{32}P up-take, rate of the up-take reaches its maximum 2 to 3 days after partial resection of the liver when the regeneration of the liver is the most prosperous, demonstrating the increased ability of phospholipid synthesis at this stage[37][38]. In the present experiment, intrahepatic total phospholipid content increased temporarily in an early stage after partial resection of the liver, and total phospholipid content in blood also showed a tendency of slight increase after surgery. From these findings, it is obviously assumed that ability of phospholipid synthesis is increased in regenerated liver and at the same time release of thus synthesized phospholipid is not hindered (Fig. 4 and 13). Total phospholipid content in blood was far higher in the group with estrogen administration than in the group without it, which might be justifiably presumed, as BALFOUR[36] pointed out, that the ability of phospholipid synthesis in the liver was strengthened by the administration of estrogen.

In the state of liver insufficiency, there develops various disorders of hormones within the entire body, and disorders due to metabolic disturbance of sexual hormones are particularly remarkable. Especially, metabolic process of estrogen in the liver has been noted since early days, and a concept of the hepato-gonadal system has come to be proposed, based on the fact that intracorporeal regulation of sexual hormones is achieved by such an organ other than the endocrine glands as the liver. The liver is the principal organ of estrogen metabolism, and disorders of liver function necessarily result in disorder of estrogen metabolism. In other words, the liver is posessed of a function to inactivate the effect of estrogen[39], and it is already demonstrated that this function of the liver essentially consists in the effect of ferments of cytochrome system within this organ as oxidase and succinic dehydrogenase. BISKIND[40] pointed out that androgen is more easily inactivated than estrogen, and, accordingly, in the state of liver insufficiency most part of estrogen remains to be active in blood stream, whereas androgen is almost entirely inactivated. It is reported that effect of estrogen is intensified in animals of experimental liver insufficiency[41], in cases of liver cirrhosis[42] and by partial resection of the liver[43].

The liver is also main tissue of cholesterol synthesis as well as phospholipid synthesis, and it is clarified at the same time by the studies with radioactive isotope that decomposition and storage of cholesterol, interchange with cholesterol in blood stream, excretion of cholesterol into bile, esterification of cholesterol and production of bile acid and steroids are exerted in the liver. TENNENT[44] observed an increase of cholesterol in blood in his perfusion experiment of heart-lung-liver preparations with acetic acid solution, while he could not demonstrate the synthesis of cholesterol when heart-lung preparation was used. From this finding, he considered the liver as the source of cholesterol supply into blood stream. Similarly, SEIFTER[31] and ECKLES[45] also attributed the source of blood cholesterol to the liver in their experiments using dogs without liver. Cholesterol content in bile is

decreased by administration of estrogen, which was interpreted by ROSENMAN[46] to be due to decrease in cholesterol synthesis in the liver.

MUKHERJEE[47] observed that the administration of estradiol in male rats resulted in decrease in cholesterol synthesis and the administration of testosterone in female rats resulted in increase in cholesterol synthesis up to the level of normal male rats. Then, he investigated influence of extirpation of the sexual gland and observed that extirpation of the sexual gland in male rats resulted in decrease in the biosynthesis to the level lower than in normal female rats, while there was no difference in biosynthesis in female rats with extirpation of the sexual gland from the level in normal rats. He reported furthermore that the administration of masculine hormone in female rats with extirpation of the sexual gland resulted in the biosynthesis of the same level as in normal male rats, while the administration of feminine hormone in male rats with extirpation of the sexual gland resulted in inhibition of the biosynthesis to the level of normal female rats. BOYD[48] also demonstrated in vitro experiment that synthesis of cholesterol in liver slice from the rats with the administration of estrogen is reduced to a half of normal rats. Moreover, it might be presumed that estrogen enhances destruction of cholesterol, since there are some reports to point out that activity of liver mitochondoria to oxidize cholesterol is stronger in female rats and male rats with estrogen administration than in normal male rats[49][50]. It might be accepted any way to be true that estrogen inhibits cholesterol synthesis in the liver. Hepatic cholesterol content increased temporarily after partial resection of the liver in the present experiment, whereas cholesterol level in blood showed a tendency of decrease after surgery (Fig. 5, 6, 7, 14, 15 and 16). It can be interpreted, as the reports of many researchers, to be due to inhibitory effect of estrogen on intrahepatic biosynthesis of cholesterol that cholesterol level in blood was invariably lower in the group of estrogen administration than in the group without estrogen administration (Fig. 14, 15 and 16).

Relationship between sexual hormone and metabolism of fatty acid is also complicated. For instance, intrahepatic biosynthesis of fatty acid is increased by castration in male rats[51], and it is inhibited by the administration of testosterone in male rats[52]. On the other hand, the administration of estradiol in female rats increases biosynthesis of fatty acid in the liver, and administration of protein anabolic hormones increases biosynthesis of fatty acid in the liver in vitro experiments[53]. Extirpation of the ovary decreases fat content within the liver as well as the administration of estrogen[2][54]. However, principal effect of androgen on lipid metabolism is a secondary one which premises metabolism of protein. In this respect, NYDEN[55] insisted that castration had little influence on biosynthesis of fatty acid in liver slice in vitro experiment. In the present experiment in which marked increase in total fatty acid content within the liver after partial resection of the liver, the degree of the increase was smaller in the group with estrogen administration than in the group without it, as shown in Fig. 10. Total fatty acid content in blood showed reversed phenomenon as shown in Fig. 19, and it is an interesting finding that hyperlipemia of considerable degree could be observed in the group with estrogen administration compared with the group without it. This finding is interpreted to suggest strongly that it is partly because estrogen is posessed of an effect to enhance release of fatty acid into blood stream and consequently that this hormone inhibits accumulation of fat in the liver.

According to FARBER[56][57], fatty liver develops in female rats by the administration

of ethionine which seldom occurs in male rats, and there exists a difference due to sexuality of the animals. Concerning sexual difference in development of fatty liver, studies have been carried out since relatively long ago. LORENZ[58] noticed influence of diet, sexuality and ovarian function on the development of fatty liver and made an experiment using cocks and hens. Based on the observation that intrahepatic neutral fat increased as hens grew older, while there was little change in phospholipid and esterified cholesterol, he asserted that the observed change of intrahepatic neutral fat was concerned with the function of the ovary. SZEGO[2] also observed that accumulation of fat in the liver is inhibited by extirpation of the ovary. On the other hand, GYÖRGY and others[59][60] made studies on fatty liver in rats kept with low protein-high fat and alipotropic diet, and they reported that lipotropic effect of ethyl estradiol is the strongest among ovarian hormones and estrone and estradiol benzoate have the similar effect, whereas masculine hormones, particularly testosterone, have no lipotropic effect. As has been surveyed, despite the fact that opinions of the researchers coincide as to sexual difference in the development of experimental fatty liver, definite opinion is not yet established in the problem that which of estrogen or androgen should be emphasized as the factor related to the development of fatty liver.

Since the reports of GORDON and others[61], it has been considered that source of N. E. F. A. supply into blood stream in the fasting state consists in the adipose tissue. Fatty acid is released into blood stream, which is produced by hydrolysis of triglyceride in the tissue, most of which exists in blood stream in a form of fatty acid bound with albumin, and it is transported to all the tissues in the body, particularly to the liver and muscles to join in the metabolic cycle. In other words, useful substances for oxidation is stored in the adipose tissue of the organism in a form of triglyceride, which is converted to N. E. F. A., when necessary, and provided to metabolism through blood stream. Although N. E. F. A. content in blood is small, its turnover proceeds so quickly that N. E. F. A. is considered to be an important form for lipid transportation as the energy source[62][63]. On the other hand, after entering the liver, N.E.F.A. forms ester together with glycerophosphate, and later appears in blood stream in a form of lipoprotein.[64][65] This triglyceride reaches the peripheral tissue through blood stream and hydrolized by lipoprotein lipase there and taken into the tissue as free fatty acid. Namely, N. E. F. A. and neutral fat in blood stream have important significance chiefly as the energy source as well as the source of fatty acid supply which is necessary for phospholipid and esterified cholesterol. As considered from kinetic aspect of fatty acid in the problem of fat accumulation in the liver, it is assumed to be a large factor in the development of fatty liver that the mechanism of release of neutral fat into the blood is disturbed.[66]

RECKNAGEL[67] considered that the disturbance of hepatic triglyceride secretory mechanism must be a principal cause of development of fatty liver, and HEIMBERG[68] presumed that disturbace of lipoprotein synthesis due to disorder of endoplasmic reticulum causes the inhibition of hepatic triglyceride transportation into blood stream, introducing the development of fatty liver. SEAKINS and ROBINSON[69] also maintained that the cause of fatty liver might be attributable to the secondary inhibition of lipid transportation from the liver to the peripheral tissue due to disturbace of lipoprotein synthesis in the liver. Based on the fact that fatty acid constitution of lipid in fatty liver resembles that of the adipose tissue, TAKAHASHI[70][71] postulated that draining of fatty acid into the liver must be an

unnegligible factor in the development of fatty liver.

N. E. F. A. content in blood fluctuates by the influence of various hormones. For instance, administration of glucose inhibits release of N. E. F. A. from the adipose tissue[72], making a reduction of N. E. F. A. content in blood and of its uptake in the liver and cardiac muscle[61)73]. Administration of insulin also inhibits, as well as administration of glucose, release of N. E. F. A. from the adipose tissue similarly[72], reducing N. E. F. A. content in blood and its uptake in the liver and cardiac muscle[61)73]. On the other side, epinephrine and norepinephrine increase release of N .E. F. A. from the adipose tissue[72], with resulting increase in N. E. F. A. content in blood and in uptake of N. E. F. A. in the liver and cardiac muscle[61)73]. Among sexual hormones, estrogen inhibits release of N. E. F. A. in vitro experiment, which is, on the contrary, enhanced by androgen[74]. Accepting the assertion that transportation of fatty acid from the peripheral adipose tissue to the liver is one of the most important factors in the development of fatty liver, SHINKO[75] maintained that testosterone enhances release of N. E. F. A. from the peripheral adipose tissue and estradiol also has this effect but in a lesser degree, and estradiol could be a cause of accumulation of fat in the peripheral tissue. Although N. E. F. A. in blood showed the tendency of increase after partial resection of the liver in the present experiment, as shown in Fig. 22, N. E. F. A. content in blood was invariably lower throughout the entire course of the experiment in the group of estrogen administration than in the one without it. This is assumed to be an interesting finding, considering together with the report[76] that successive administration of estradiol causes the tendency of decrease in N. E. F. A. content in blood. Moreover, neutral fat content in blood was contrariwise higher in the group with estrogen administration than in the group without it. From this fact, it is interpreted that estrogen inhibits release of N. E. F. A. from the peripheral adipose tissue and enhances release of neutral fat from the liver to blood stream.

Concerning the influence of sexual hormone on liver regeneration, KOCHAKIAN[77] maintained that decrease in liver weight and liver RNA content following castration can be improved by anabolic effect of testosterone propionate, androstanolone and methyltesterone. HALL[78] reported that administration of oestrone or oestradiol in castrated rats resulted in decrease in liver weight compared with liver weight in normal rats. Concerning regeneration of the residual liver parenchyma after partial resection of the liver, it is reported, on the other hand, that castration inhibits regeneration of the liver[5] and administration of testosterone propionate enhances it in male rats[4)5].

As has been surveyed, effect of sexual hormones is subtle and complicated depending upon sexuality. In the present experiment, there could be observed little significant difference between the group with estrogen administration and the one without it as to the results of female rats, being different from those of male rats, as shown in Fig. 23 and 24. Namely, as far as male rats are concerned, it was disclosed in the present experiment that estrogen is posessed of an effect to enhance restoration of the residual liver weight after partial resection of the liver, and it was further suggested that estrogen is possessed of an extremely favorable influence on liver regeneration, though the mechanism of its action could not be clarified.

V. SUMMARY

Influence of estrogen on liver regeneration and lipid metabolism following partial resection of the liver was studied in comparison with the animals without administration of estrogen.

1) Increase in body weight after partial resection of the liver was more rapid in the group with estrogen administration than the one without it, showing restoration of body weight to the preoperative level to be 5 days after surgery.

2) The rate of increase in liver weight after partial resection of the liver was extremely large in the group with estrogen administration as compared with the group without it, and the difference of the rate between these two groups is most remarkable 2 to 3 days after surgery when liver regeneration is at its maximum.

3) Each fraction of intrahepatic lipids showed the maximum level 10 hours after partial resection of the liver, which was followed by restoration to the preoperative level 5 days after surgery. Every fraction of the lipids showed invariably lower level in the group with estrogen administration compared with the group without it, which was particularly marked in the fractions of intrahepatic total fatty acids, neutral fat and total lipids.

4) Intrahepatic cholesterol-ester ratio and total cholesterol/total phospholipids ratio showed slight increase temporarily after partial resection of the liver. However, there was little significant difference between the both groupe with and without estrogen administration.

5) Lipid fraction in blood, particularly total phospholipids and neutral fat showed a tendency of increase after partial resection of the liver, whereas total cholesterol and esterified cholesterol decreased contrariwise. On the other hand, cholesterol of free type, total fatty acids and total lipids showed little fluctuation throughout the pre- and postoperative period.

6) In the group with estrogen administration, contents of total phospholipids, total fatty acids, neutral fat and total lipids in blood were considerably higher than in the group without estrogen administration. However, the relationship was reversed as to the contents of total cholesterol, esterified cholesterol and cholesterol of free type.

7) Cholesterol ester ratio and total cholesterol/total phospholipids ratio in blood showed a tendency of decrease after partial resection of the liver, the ratios invariably lower in the group with estrogen administration than in the one without it throughout the entire course of the experiment.

8) N. E. F. A. content showed a tendency of increase after surgery in both groups with and without estrogen administration. However, the content was constantly smaller in the group with estrogen administration than in the one without it.

9) Histological study revealed no significant difference between the both groups with and without estrogen administration.

10) From these findings of the present experiment, it was clarified that administration of estrogen enhances regeneration of the residual liver parenchyma after partial resection of the liver and inhibits accumulation of fat within the liver. In other words, it is assumed that such effects of estrogen can be pointed out as 1) to inhibit release of N. E. F. A. from the peripheral adipose tissue, 2) to inhibit synthesis of cholesterol within

the liver and 3) to enhence release of neutral fat into blood stream which are the results of increased synthesis of phospholipid within the liver.

Accomplishing the present paper, the author is deeply indebted to Prof. Dr. Ichio Honjo for his enthusiastic guidance and valuable advices.

The gist of the present paper was reported at 39th Annual Meeting of Japan Endocrinological Society and 45th Conference of Surgeons in Kyoto.

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(* in Japanese)

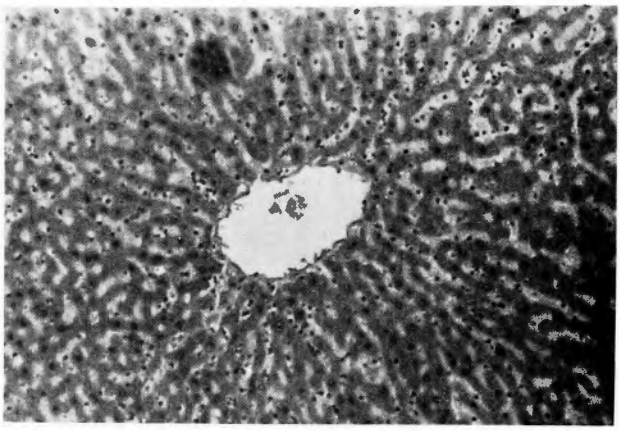

Fig. 25 Liver of non-treated rats 10 hours after partial hepatectomy.

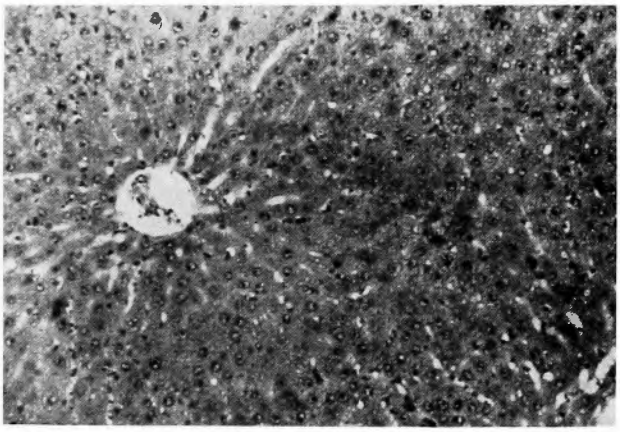

Fig. 26 Liver of estrogen-treated rats 24 hours after partial hepatectomy.

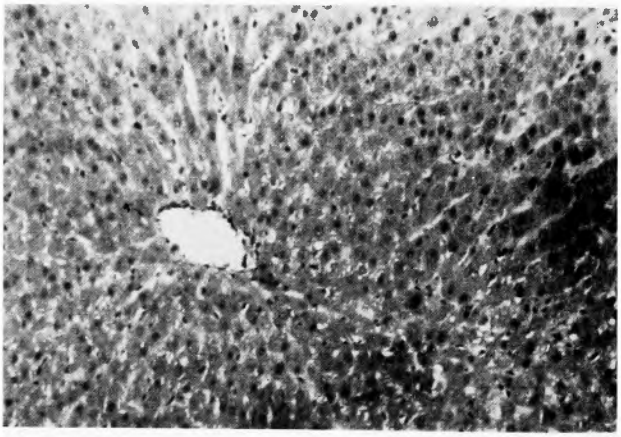

Fig. 27 Liver of non-treated rats 2 days after partial hepatectomy.

和文抄録

肝部分切除後の脂質代謝に及ぼす Estrogen の影響

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肝の再生力の旺盛な事実は古くから報告されており，Higgins & Anderson によれば，ラットで肝70%切除しても，3週間以内に肝重量は正常に復するという．肝が旺盛な再生活動を営むに当り，脂質が積極的に関与し，Szego は，肝切除後の再生初期の肝重量増加は脂質の増加によるとし，青山等は，脂質がその円滑な再生過程の必須要素であるとした．しかし，Bengmark 等は Testosterone propionate の投与は，肝切除後の残存肝の再生を促進し，しかも肝脂肪浸潤を抑制するとした．かかる意味で，肝再生初期の肝脂肪の増量は，必ずしも肝再生機能亢進の現れとは考え難い．性腺ホルモンの中，脂質代謝と密接な関連にある Estrogen を投与して，肝切除後の肝再生，並びに脂質代謝に及ぼす影響を検討した．

体重100～160 g 雄ラットを用い，Higgins & Anderson の肝部分切除法に従い，エーテル麻酔下に肝62%切除した．術後10時間 1，2，3，5 日と経時的に心穿刺により採血屠殺後，肝を剔出，ただちに実験に供した．Miescher の報告より，Estrogen を体重100 g 当り10μg筋注後，7日目に肝切除術を施行した．

1) 術後の体重変動　Estrogen 投与群及び非投与群の，肝切除後の体重の回復・増加の割合を比較検討するに，前者はその回復・増加が速やかである．

2) 再生肝重量の変動　肝切除後早期より，残存肝は旺盛な再生肥大を示し，Estrogen 投与群の肝重量増加の割合は，非投与群に比し遙かに大であり，術後2日乃至3日目に顕著である．

3) 肝脂質の変動　肝切除後，肝脂質分画は急激に増量し，術後10時間で夫々最高値を示し，3日乃至5日目でほぼ術前値に回復する．総燐脂質は両群有意の差なく，Cholesterol 値は Estrogen 投与群が全経過を通じ低値を示した．総脂肪酸・中性脂肪・総脂質は術後早期に著明に増量し，就中，中性脂肪では非投与群の術後10時間値が，術前値の約6倍に増量した．Estrogen 投与群では，3者とも術後の増量は軽度で且つ回復も速やかであつた．肝エステル比，c/p 比は術後軽度増加するも，両群有意の差を認めない．

4) 血中脂質の変動　血中脂質分画中，総燐脂質及び中性脂肪は術後増加傾向を示すのに対し，Cholesterol は減少した．Estrogen 投与群は，総燐脂質・総脂肪酸・中性脂肪・総脂質で非投与群よりかなりの高血中濃度を示すも，Cholesterol では逆の現象を呈した．血中エステル比，c/p 比は術後減少傾向を示した．血漿 NEFA は，両群とも術後増加傾向を示すも，Estrogen 投与群が非投与群より常に低値を示したのが注目される．

5) 再生肝の組織像　両群有意の差を認めない．

以上の実験成績より，Estrogen の投与は肝切除後の残存肝の再生を促進させ，肝への脂肪蓄積を抑制した．脂肪肝の成因につき，末梢脂肪組織から肝への fatty acid transport，並びに Recknagel 等のいう肝中性脂肪分泌機構の障害が重要な因子であるという説に従えば，Estrogen には，1) 末梢脂肪組織からの NEFA の放出を抑制し，2) 肝での Cholesterol 合成を抑制する事，及び 3) 肝内での，燐脂質合成能亢進による燐脂質及び中性脂肪の血中への放出を盛んにする等の諸種因子が考慮される．